PRELIMINARY NOTE ON THE SPERMATOGENESIS OF PEDICULUS VESTIMENTI.[1]

KATHARINE FOOT.

In response to a request from Col. Alexander Lambert I came to Paris, December, 1917, in order to study *Pediculus vestimenti* from the point of view of the biologist in the hope of getting some data that might be of service to the investigators who were studying the louse as a possible transmitter of certain diseases prevalent among the troops.

In an exhaustive and masterly study of the problem in relation to trench fever, Col. Strong has shown that the lice can unquestionably transmit this disease and has proved beyond question what was heretofore merely a surmise.

Such data as I have been able to collect that may have some bearing on the problems in relation to disease have been reported to the Research Department of the American Red Cross; but I have omitted from such reports purely cytological data which have no apparent practical value.

As far as I know, no report has been published of any work done on the chromosomes of the louse, and as *P. vestimenti* has been classed as an Hemipterous insect I was interested to see if the chromosomes have the same bizarre morphological characteristics as are typical of so many Hemiptera.

The spermatocyte chromosomes are very minute, so minute that I have as yet found it impossible to demonstrate the method of division of the second spermatocytes; but I have several entirely satisfactory stages of the first spermatocyte chromosomes, and as these have the same morphological characteristics as the corresponding stages in other species of Hemiptera, it is quite permissible to assume a like correspondence for the second division.

[1] My grateful acknowledgments are due to the late Professor Blanchard, who gave me a most cordial welcome to his laboratory at the Ecole de Médecine. He not only gave me ample space for my work but the sympathy and encouragement I received from him and the members of his staff made it possible for me to continue my investigations of these repulsive insects.

The above mentioned morphological peculiarity of the chromosomes of many Hemiptera *i. e.* the unequal bivalent—called by Wilson the X Y chromosome—is present in the first spermatocytes of *Pediculus vestimenti* and its division is typical—the two unequal parts dividing as univalents in the first division. The unequal bivalent is demonstrated in the metaphase stages of Figs. 10, 11, 12, and the division of the smaller half of the unequal bivalent is shown in Fig. 13.

Miss Strobell and I (1914) published a series of photographs showing both the first and second spermatocyte divisions in *Euschistus variolarius*, *Euschistus servus* and in two generations of hybrids from *E. variolarius* by *E. servus*. A comparison of these photographs of the first spermatocyte chromosomes with the above mentioned Figs. 10–13 will show that the first spermatocyte chromosomes of *Pediculus vestimenti* are of the same type as in the species of *Euschistus* referred to, and it is therefore quite logical to assume that the second spermatocyte chromosomes are equally typical though its demonstration is not yet possible.

In Fig. 16 (on the right) is a group of chromosomes from an embryo in an egg at the basal end of the ovary.

It is not possible to determine the exact number of chromosomes of this group, as 18, 19 or 20 can be counted.

The first spermatocyte chromosomes indicate that the somatic number should be ten and in the few spermatogonial groups I have found it possible to identify ten chromosomes; but they are so small and so frequently constricted that the estimate can always be questioned.

I am convinced that the most favorable stage for an exact interpretation of the louse chromosomes is the first oöcyte prophase, but to secure this stage involves a patient search which cannot be undertaken at present. Fig. 18 indicates that it will be possible to find the later prophase stages, for in this preparation the chromosomes are nearly formed, and it ought to be possible to find the slightly later stages in the same locality of the ovary.

An early stage of the yolk spheres of the ovary is shown in the photomicrograph of Fig. 16 (on the left). These are chromosome-like structures, which in the early stages select the chroma-

tin stains and are morphologically so like chromosomes that one is tempted to interpret them as chromosomes which develop into yolk-spheres.

A series of stages prior to the first spermatocyte metaphase is shown in Figs. 1 to 9. The centrosomes of the first spermatocytes (Figs. 1 to 4) are a feature of these stages which is most marked. The position of these centrosomes in relation to the nucleus is very variable: they are in contact with the nucleus or almost in contact with the periphery of the cell or in any position between these two extremes. In later stages they are frequently divided into two or more, rarely into three, parts. Several of the former are shown in Fig. 7 (the centrosomes of this figure are a little exaggerated by the artist).

The Development of the Spermatid into the Mature Spermatozoön. —The most striking feature in the development of the spermatozoön is the duplex character of the tail. Apparently the tail is composed of two distinct and independent filaments, the apparent independence of these filaments being more marked in the earlier stage (Figs. 20–21). One would naturally suppose that one of these filaments represents the flagellum of other forms, but it has not been possible to demonstrate any substance connecting the two filaments, though the fact that even in smear preparations the two are never found widely separated would indicate that they are attached by some connecting substance.

Historical Sketch.

Certainly no insect has been accused of being the promoter of a greater variety of diseases than the louse and perhaps no accused has been charged with so many crimes on less evidence. *Pediculus* has been credited with transmitting the following diseases: typhus fever, typhoid fever, recurrent fever, trench fever, tuberculosis, spinal meningitis, plague, leprosy, beri-beri and more than a dozen minor skin diseases. In some of these cases the evidence seems to be confined to the fact that the patient may be infested with lice.

For typhus fever, recurrent fever and trench fever it has been proved that the lice do in fact transmit these diseases, but details as to the method of transmission are still disputed. It is held

by many investigators that germs are not transmitted by the bite of the louse—the sole method of transmission being infection from their excrements. These are freely deposited on the skin and in the clothing of the host and subsequent scratching of the skin induced by the intense itching of the bites not only lacerates the surface but frequently causes a deep wound that leaves a scar lasting many months. It is self-evident that such lacerations over surfaces more or less infested with the excrements of the lice may cause a most effective inoculation. This method of infection by the fæces or the crushed body of the louse has been demonstrated for typhus fever by Nicolle (1909), Nicolle, Conte and Conseil (1910) and others. For recurrent fever by Sergent and Foley (1910), Sergent, Gillot and Foley (1911), Nicolle, Blaizot and Conseil (1912) and others. For trench fever, by R. P. Strong (1918). Familiarity with the feeding habits of the louse demonstrates the danger of this method of inoculation, for the amount of excrement discharged by each louse is surprising. I have frequently seen a single louse, during one hour's feeding, discharge excrement ten times, and five times is not unusual. For more than a year I have closely studied the feeding habits of *Pediculus vestimenti* and in my report to the research department of the Red Cross I described their behavior as follows: "Observations made during the feeding hour demonstrate that individual lice may behave very differently. As a rule they bite at once when young and vigorous. Some become gorged with blood in ten minutes and will not bite again, though most frequently they bite several times during the hour, moving around rather restlessly between times. Others bite continuously the entire hour, casting their excrements while biting. The old lice frequently do not bite for several minutes or even half an hour and then suck the blood very deliberately." I am inclined to believe that the method of biting demonstrated for one hour indicates the method for the entire twenty-four hours and that therefore the younger lice are almost continuously feeding on the host, wandering about and biting very frequently.[1] This would accord with observations made by Miss Strobell and

[1] These observations support Nuttall's (1917) conclusions as to the probable feeding habits of lice. He thinks they bite very frequently, for when raising them on his wrist he noticed they started to bite at all times when he was quiet.

myself on other species of Hemiptera, *Euschistus variolarius*, *E. servus*, *E. ictericus*, etc., etc. These insects were fed on fruit and could be closely observed during the twenty-four hours. They fed almost continuously during the night as well as during the day, and this leads me to surmise that the lice may feed with equal frequency and explains the torment that soldiers suffer even when infested with relatively few lice and suggests the possibility of inoculation from a single louse.

The disputed question whether simply the bite of the louse can inoculate the host has given rise to much discussion, some investigators emphatically denying that any danger is caused by the bite alone. Colonel Strong (1918) conducted some experiments with the aim to determine this point and concludes that "it seems fair to argue that the bite is probably a common mode of infection." He states that in some instances the disease was produced by pure biting experiments. His summary of the probable methods of infection through biting is as follows:

1. By piercing or stabbing and inoculating with mouth-parts contaminated with infected material such as blood from the patient or by louse fæces and body juices.

2. By stabbing and inoculating from the skin which has been contaminated with infected material such as louse fæces, and possibly body juices.

3. By stabbing and inoculating with mouth-parts which have been contaminated with virus grown or developed in the stabber-sac.

4. By stabbing and regurgitating of the virus from the alimentary canal.

5. By stabbing and the injection of the virus contained in the salivary juices.

6. By hereditary infection.

One of the difficulties in determining the value of the bite alone is to eliminate the fæces from the experiment. Those cases in which this is assumed to be done by allowing the louse to bite through chiffon do not appear to me to be conclusive, for in my experience they will not bite unless the chiffon is pressed upon the surface with sufficient strength to force the skin through the interstices of the chiffon, in which case the

only effect of the chiffon is to reduce the area of the skin exposed, and the lice wander over the exposed area distributing the fæces as usual.

The danger of the louse as a promoter of disease has been so long appreciated that he has claimed the attention of a large number of investigators, the French and English forming the majority. The work accomplished up to 1917 has been most ably presented by Professor Nuttall, of Cambridge. His bibliographical list is an index of the thoroughness of his historical study of the subject. He has listed nearly 600 investigators.

A second historical sketch was published in France the same year (Souéges et du Noyer, 1917). These two studies are a convenient record of all the historical data that can be of value to the investigator.

Two English investigators (Warburton, 1909, and Fantham, 1912) were the first to study the life history of *Pediculus* and their results were supported and extended by Bacot in 1916. He determined the number of moults to be three, the average length of life of the louse, the average number of eggs deposited daily by a single female and other details, all of which my investigations support although our methods of work differed materially. He used an entomological box containing a number of lice and strapped this box on his person each night, allowing the lice to bite from six to seven hours daily. My lice were fed only one hour in the twenty-four and in such a manner that I could watch them while feeding.

The most serious difficulty in the investigation of lice is the food supply. In all the accounts with which I am familiar the investigator has had sufficient self-abnegation to feed his lice on his own person, but not having reached those heights myself, my initial difficulty was to find a host. There seems to be something extremely ridiculous in the mere suggestion of feeding a louse, for my most serious and generous offers received the discouraging response of a broad grin and an emphatic shake of the head. I finally succeeded in securing a host at the Asile de Nuit—a night employee of that institution. He was an old sailor whose evident familiarity with *Pediculus* at the Asile de Nuit had led him to cease to regard them in a humorous light,

and he proved to be a thoroughly dependable food supply. He never missed his daily hour in my laboratory for the five months I employed him.

When feeding the lice I at first used the usual method of putting a number in a tube, inverting the tube on the arm and holding it securely in place to prevent the lice from escaping. I found this method unsatisfactory for several reasons and devised therefore quite a different technique. Lice cannot crawl up a glass surface if it is clean and are therefore perfectly safe in a glass ring even if it is only 2 cm. high. I had such rings made to order and fastened them securely onto the arm with melted paraffine. In this manner several different experiments can be conducted at the same time and the generations can be kept separate—further the lice can be conveniently studied with a lens during the hour they are feeding. For the remaining twenty-three hours they were kept in a Pasteur incubator at a temperature between 27° and 29° C. While in the incubator the lice were kept in cages such as those used in the laboratory for raising various insects. This cage is the tube de Borel, in which is placed an inner tube for the insects, this being held in the center by absorbent cotton which is kept wet to insure sufficient moisture. I found the use of absorbent cotton very inconvenient and replaced it with a short tube having an aperture large enough to contain the inner tube and open at both ends with a lip at each end sufficiently wide to center it in the tube de Borel. The inner tube in which the insects are kept is dropped into this shorter tube and an inch of water kept in the tube de Borel. I found this method a great economy of time, for it was necessary to pack the cotton around the inner tube with much care, since if the opening came in contact with the inner surface of the tube de Borel (often quite wet) a drop or two sometimes dripped into the inner tube and cost the life of one or more nymphs.

Several years of experience in crossing and raising other species of Hemiptera have been my guide in raising the lice. Miss Strobell and I found that the species we studied required as much humidity as possible while avoiding any condensation of the moisture. This I have found true for lice—a half a drop of water or less can kill a nymph. If he gets on his back on the

glass in even a fraction of a drop of water, he cannot regain his feet until the water dries and if the glass is not clean he adheres to it and finally dies.

After trying the usual method of keeping the lice on small pieces of woollen or muslin cloth it occurred to me that a large number of short pieces (about 8 mm.) of soft, coarse thread would have many advantages. First they would be much more sanitary for they can be changed every day if necessary without disturbing the lice at all. When lice are on a small piece of cloth, the cloth becomes filthy in a few days and it is exceedingly difficult to remove the lice to fresh pieces. Further the thread avoids all the difficulties encountered in transferring and counting the lice. They cling to a thread with great tenacity; therefore single lice can be carried on a thread any distance with perfect safety. They deposit their eggs on the thread and therefore the eggs deposited each day can be conveniently collected and isolated. Using these threads made it a simple matter to record the following life history of a single pair of lice. The pair was hatched from eggs deposited in the laboratory and had their third (final) moult August 19. They were seen mating August 22. The next day 4 eggs were deposited and thereafter 4, 5 or 6 were deposited daily until the female died.

RECORD FL. D.

1918.		Eggs.	Hatched.
Aug.	(Mated Aug. 22)		
23	Mated	4	
24		3	
25		5	
26		4	
27		4	
28		5	
29		5	
30		4	
31		5	
Sept.			
1		5	
2	Hatched the 10th day	6	1
3		5	3
4		5	4
5	(Mated)	5	3
6	(Male dead—each daily deposition of eggs kept separate from death of male)	5	7
7		5	3

1918.		Eggs.	Hatched.
Sept.	(Mated Aug. 22)		
8		6	5
9		5	5
10		5	5
11		6	4
12		5	5
13		5	6
14		5	4
15	Female dead	1	5
		113	
16			5
17	5 hatched from eggs deposited 9–7 1 " " " " 9–8		6
18	3 hatched from eggs deposited 9–8 1 " " " " 9–9		4
19	2 hatched from eggs deposited 9–8 3 " " " " 9–9 1 " " " " 9–10		6
20	1 hatched (the last) of eggs deposited 9–9 3 " from eggs deposited 9–10 1 " " " " 9–11 2 " " " " 9–12		7
21	1 hatched (the last) of eggs deposited 9–10 5 " (the last) " " " 9–11 2 " from eggs deposited 9–12 3 " " " " 9–13		11
22	2 hatched (the last) of eggs deposited 9–13 2 " from eggs deposited 9–14 1 " " " " 9–15		5
23	3 hatched (the last) of eggs deposited 9–14 The last egg deposited 9–12 not hatched, nymph partly emerged but dead.		3

SUMMARY.

If we omit the one egg deposited the day the female died the daily average for the remaining 112 eggs is 4.9.[1]

113 eggs were deposited from August 23 to September 15 and all of these developed to the nymph stage, though six failed to completely emerge from the egg.

The above record shows that the male died September 6 and the female died September 15. From September 6 each daily deposition of eggs was isolated to determine how long the eggs were fertilized after the last mating (September 5). The record shows that all the eggs developed and that all hatched but one.

[1] Bacot found the daily average to be 5.1 and Nuttall's experiments demonstrated that under natural conditions, *i.e.*, when the lice lived continuously on the host, the daily average increased to 9.7.

As the female had her third moult between the 18th and 19th of August, she lived only 28 days after maturity, less than the average length of life for a female; but other females in the laboratory kept under the same conditions lived 42 days, 40 days, 39 days, etc. Bacot found the average length of life of a female to be 34 days. If my lice are a little below this average I think it is probably due to the difference in feeding—he fed his lice six or seven hours daily and mine were fed only one hour daily.

According to my experience, the longer a race is bred in the laboratory the less prolific they become and the death rate is much higher. I believe this is due entirely to an abnormal lack of nourishment. Feeding only one hour in twenty-four is certainly very abnormal for these insects.

One cannot study the record of the large amount of experimental work done on the louse without being impressed with the need of feeding these insects apart from the human host before certain problems now in dispute can be solved. My efforts have been largely given to this well high hopeless task which is my apology for a very superficial study of the spermatogenesis.

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EXPLANATION OF PLATES.

All the sketches were drawn at a magnification of about 750. Zeiss hom. immer. 2 mm. 140—apo. oc. IV camera lucida.

TECHNIQUE.

Smear preparations stained with May-Grünwald followed by Hollande.

These stains were used in accordance with the method published by Dr. Langeron (1916) and I am greatly indebted to him for many valuable suggestions and encouraging interest in my work.

PLATE I.

FIGS. 1, 2, 3, 4. First spermatocyte rest stages, each showing a distinct centrosome in varying proximity to the nucleus. No cell membranes are differentiated.

FIG. 5. First spermatocyte nucleus showing a single nucleolus and the chromatin segregating to form the chromosomes.

FIG. 6. Two first spermatocyte nuclei with granular chromatin segregating to form the chromosomes. The nucleolus has disappeared.

FIG. 7. Numerous first spermatocyte nuclei showing successive stages of the differentiation of the chromatin. In the earlier stages the chromatin is apparently homogeneous and later it is granular and segregating into definite masses to form the chromosomes. A few centrosomes are differentiated.

FIG. 8. Numerous first spermatocyte nuclei showing later stages than those of Fig. 7. In many of the nuclei the chromatin has segregated into 5 distinct masses foreshadowing the 5 bivalent chromosomes of the first spermatocyte metaphase.

FIG. 9. First spermatocyte nuclei about the same stage of development as those of Fig. 7.

FIGS. 10, 11, 12 and 13. Each figure shows the 5 bivalent chromosomes of the first metaphase. In each figure one or more of the chromosomes is a dyad, foreshadowing the first division. An unequal bivalent, which is typical of so many Hemiptera, is clearly shown in each group.

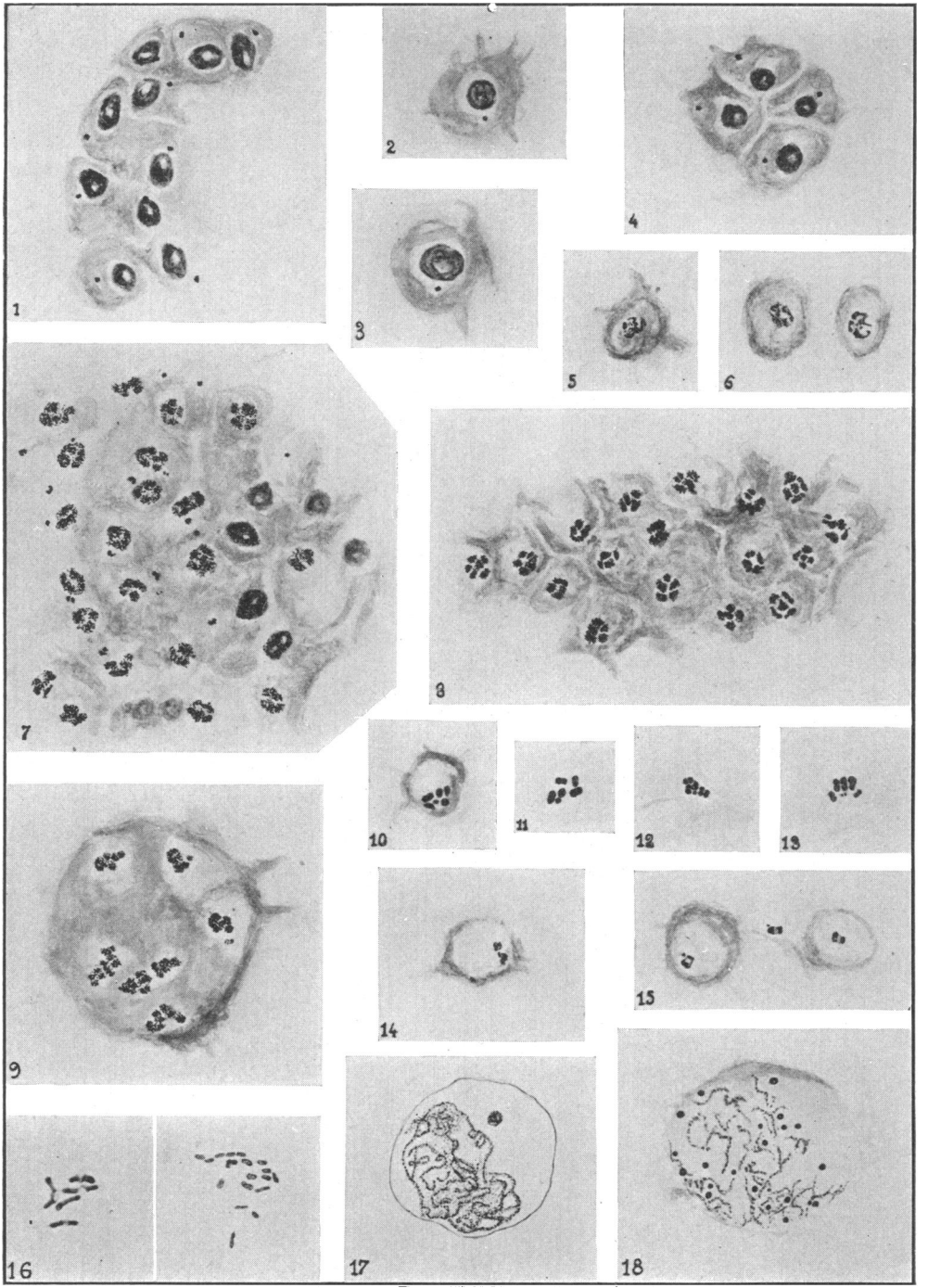
FIG. 13. All the chromosomes of this first metaphase group are dyads, foreshadowing the division of each. The large and small chromosomes of the unequal bivalent are detached and each is a dyad. This indicates that each will divide in the first division and that therefore the resulting halves will undoubtedly separate in the second division in the manner typical of so many Hemiptera.

FIG. 14. Late anaphase of the second division. The chromosomes are too small and too closely segregated to determine their number and form.

FIG. 15. Three telophases of the second division. Each shows an unequal division of the chromatin, this being the sole evidence, at this stage, of the separation of the large and small moieties of the unequal bivalent.

FIG. 16. On the left a photomicrograph of a small group of chromosome-like structures from an immature ovary. From these the yolk-spheres are developed. $\times$ 450. On the right a sketch of a group of chromosomes from an embryo in an egg at the basal end of the ovary.

FIGS. 17, 18. Two germinal vesicles from young ovarian eggs. In Fig. 17 the chromatin has partly segregated into threads, and a single nucleolus is present. Fig. 18 shows numerous small dense nucleoli, and the separate chromatin threads suggest a progressive step in the forming of the chromosomes though their abnormal number may be due in part to the technique.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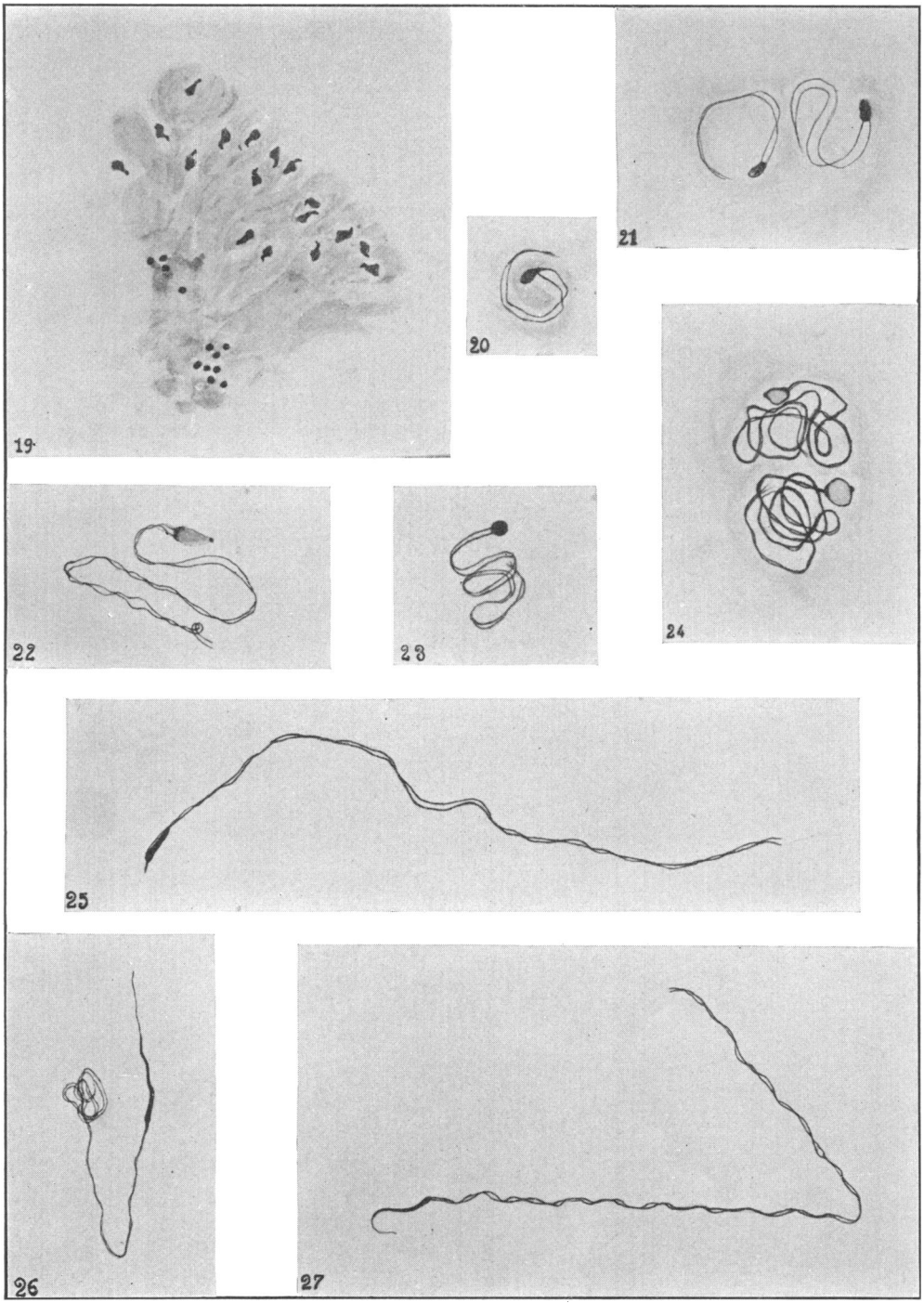
EXPLANATION OF PLATE II.

FIG. 19. Numerous heads of spermatids shortly after the second division. They are at first round, as demonstrated in the figure, and, as a rule, elongate before the appearance of the tail. In the elongated heads of this figure the middlepiece (?) is in evidence and from this point the tail develops.

FIGS. 20 to 27. Successive stages of the development of the spermatid into the mature spermatozoön. In Figs. 20 and 21 the middlepiece is demonstrated, the tail developing from this point. In Figs. 22 and 24 both the middlepiece and the acrosome (?) are demonstrated, the spine developing from the latter. Fig. 25 shows an early stage of the development of the spine. Figs. 26 and 27 demonstrate the mature spermatozoön in which the head and spine are fully developed. At this stage the middlepiece and acrosome are obscure.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